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The B-vitamin and Nitrogen Requirements of *Bacteroides succinogenes*, an Important Ruminal Cellulolytic Bacterium 1/

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Previous work showed that *B. succinogenes* required a fermentable carbohydrate, CO_2, and a two-component system of certain branched-and straight-chain volatile fatty acids for growth (Bryant and Doetsch, J. Dairy Sci., 38). Whether or not any of the other ingredients of the growth medium-minerals, purines and pyrimidines, enzymatic hydrolysate of casein, cysteine, and B-vitamins are growth requirements is presently being studied.

The study has been carried out using five strains, four isolated in 1953 and one fresh isolate. Table 1 shows the composition of the inoculum medium. The medium is in equilibrium with a gaseous phase of CO_2. In the early part of the work this medium also contained adenine, guanine, uracil, xanthine, thymine, Zn SO_4, Cu SO_4 and Na MoO_4 but deletion of these substances had no effect on growth. Inoculum consisted of 0.1 ml of a cell suspension prepared by centrifuging out the cells of a 16-hour culture, decanting the supernatant, and adding sterile dilution solution to an optical density of 0.1.

Table 2 shows some of the results obtained in vitamin studies.

None of the strains grew when all vitamins were deleted from the medium. When single vitamins were deleted, it was shown that biotin was a strict requirement of all strains except S121 which was greatly stimulated by this vitamin. p-Aminobenzoic acid was stimulatory to strains S61 and CB40. None of the other vitamins were required or stimulatory when single deletions were made. Optimum growth was obtained with three strains in medium with only biotin added and optimum growth of the others (S61 and CB40) was obtained with only biotin and p-aminobenzoic acid added.

Data in table 3 show that 18L-amino acids similar to those in casein promoted growth similar to that obtained with casein hydrolysate. NH_4^+ was required for good growth even when 19 amino acids, purines and pyrimidines and all of the B-vitamins were present in the medium. All strains grew, but growth was somewhat delayed, when cysteine or glutathione and NH_4^+ were the sole sources of nitrogen in the medium. One strain, CB40, grew quite poorly under these conditions.

1/ Paper presented at the annual meeting of the American Dairy Science Association, June 17-19, 1958 at North Carolina State College, Raleigh, N. C.

Table 1 - Composition of inoculum medium used to study nutritional requirements of *B. succinogenes*

	Mg./100 ml.		Mg./100 ml.
KH_2PO_4	90	Folic acid	0.005
NaCl	90	Cobalamin	0.0005
$(NH_4)_2SO_4$	90	$FeSO_4 \cdot 7H_2O$	4.0
$CaCl_2$	9	$MnSO_4 \cdot H_2O$	0.15
$MgSO_4$	9	$CoCl_2 \cdot 6H_2O$	0.15
Thiamin·HCl	0.2	Resazurin	0.10
Pyridoxamine·HCl	0.1	Casein hydrolysate 1/	200
Ca-pantothenate	0.2	Glucose	300
Riboflavin	0.2	Cysteine·HCl	100
Nicotinamide	0.2	Na_2CO_3	400
p-Aminobenzoic acid	0.01	n-Valeric acid	6.12
Biotin	0.005	Isobutyric acid	2.64

1/ "Vitamin-free" enzymatic hydrolysate. The medium was in equilibrium with CO_2 gas.

Table 2 - The effect of deletion of vitamins from the inoculum medium on growth of B. succinogenes

Vitamins Added	Growth (O.D. X 100)				
	S85	M13	S121	S61	CB40
All	96(24)[1/]	105(24)	82(24)	96(24)	92(27)
All but biotin	1(24)	1(24)	28(48)	3(24)	3(24)
All but p-aminobenzoic acid	93(24)	102(24)	79(24)	52(48)	44(112)
Biotin and p-aminobenzoic acid	92(24)	100(24)	82(24)	96(27)	82(27)
Biotin	96(24)	105(24)	80(24)	17(27)	7(24)

1/ Figures in parentheses are hours of incubation for maximum reading.

Attempts to obtain optimum growth of the organism in media containing various mixtures of amino acids have been very erratic and no definite conclusions have been made. It seems probable that many of the erratic results have been due to amino acid inhibitions and antagonisms.

Preliminary attempts to find a good reducing system to replace cysteine or glutathione in the medium so that cysteine and sulfur requirements can be studied have been unsuccessful. Levels of ascorbic acid and sodium sulfide that reduce the medium are inhibitory.

The fact that B. succinogenes grows quite well in a medium containing only minerals, valeric acid, isobutyric acid, biotin, p-aminobenzoic acid, glucose and cysteine indicates that this important ruminal cellulolytic bacterium has a relatively highly developed ability to synthesize cellular constituents.

Table 3 - The effect of some nitrogen sources on growth of *B. succinogenes*

Ingredients Added to Basal Medium	Growth (O. D. X 100)				
	S85	M13	S121	S61	CB40
Experiment 1 [1/]					
Casein hydrolysate, $(NH_4)_2SO_4$	100(24)	100(24)	96(24)	96(40)	90(40)
18L-amino acids, $(NH_4)_2SO_4$	82(24)	96(24)	96(40)	88(40)	92(40)
18L-amino acids, Na_2SO_4	4(51)	5(24)	3(51)	2(95)	3(68)
$(NH_4)_2SO_4$	77(68)	77(51)	80(68)	80(76)	1(68)
Na_2SO_4	0(119)	0(119)	0(119)	0(119)	0(119)
Experiment 2 [2/]					
Casein hydrolysate + cysteine	110(25)	105(18)	89(18)	96(40)	96(25)
Cysteine	100(42)	89(50)	82(50)	105(40)	40(108)
Glutathione	100(42)	82(42)	82(42)	96(40)	60(42)

1/ The basal medium was that shown in table 1 except that casein hydrolysate and $(NH_4)_2SO_4$ were deleted and adenine, guanine, uracil, xanthine and thymine were added.

2/ The basal medium was that shown in table 1 but minus casein hydrolysate, cysteine and all vitamins except p-aminobenzoic acid and biotin.